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# COURTSHIP, COPULATION, AND SPERM TRANSFER IN *LEUCAUGE MARIANA* (ARANEAE, TETRAGNATHIDAE) WITH IMPLICATIONS FOR HIGHER CLASSIFICATION

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**ABSTRACT**. The courtship behavior of male *Leucauge mariana* (Keyserling 1881) spiders that occurred both prior to and during copulation is described, along with the positions and movements of the male genitalia. The great variation in male behavior suggests that it does not function in species recognition. Several kinds of female response are necessary if a male is to successfully inseminate her. Males made two types of insertion, involving different movements of palpal sclerites, and copulations with virgin females differed quantitatively and qualitatively from those with non-virgins. Males deposited encapsulated sperm and other material in an outer chamber of the female's spermatheca early in copulation. Later stages of copulation involved deposition of material on the surface of the female's epigynum that sometimes resulted, with the apparent addition of material by the female, in the formation of a plug on the epigynum. Sperm were decapsulated in the female soon after insemination, perhaps as a result of the action of a female glandular product, and later were found in two other chambers of her spermathecae. Contrary to previous discussions, male and female cheliceral clasping behavior accompanying copulation does not explain why the palpal morphology of these spiders is relatively simple. Cheliceral clasping was similar, though not identical, to that of several other tetragnathine spiders. Cheliceral clasping and details of how male palps engage the female may provide synapomorphies linking *Leucauge* to tetragnathines.

**RESUMEN**. Se describe el comportamiento de cortejo de machos de *Leucauge mariana* (Keyserling 1881) que ocurrió antes y durante la cópula, y las posiciones y los movimientos de la genitalia del macho. La gran variación en el comportamiento de los machos sugiere que ésto no funciona como señal de reconocimiento de la especie del macho. Varias respuestas de las hembras son necesarias par que un macho logre inseminarla exitosamente. Los machos efectuan dos tipos de inserción de los palps en los cuales realizaron diferentes movimientos con los escleritos del palpo. Las cópulas con hembras virgenes diferieron tanto cuántitativamente como cuálitativamente de las cópulas con hembras no virgenes. Los machos introdujeron espermatozoides encapsulados y otras sustancias en una cámera de la espermateca de la hembra durante una etapa temprana en la cópula. Después depositaron materiales sobre la superficie del epígeno que a véces formaron, en combinación con material proveniente de la hembra, un tapón sobre el epígeno. Los espermatozoides salieron de las cápsulas dentro de la hembra, quizás como resultado de la acción de un producto glandular de la hembra, y después llegaron a dos otras cámeras de la espermateca. Al contrario de algunas discusiones previas, el agarre entre los quelíceros del macho y la hembra no explica porqué la morfología de los pedipalpos del macho de este grupo es relativamente sencilla. El agarre entre quelíceros se asemeja al agarre de varias otras especies de Tetragnathinae, y este comportamiento, mas otros detalles de como los palpos del macho se acoplan con la genitalia de la hembra, pueden proveer sinapomorfías que ligan *Leucauge* a Tetragnathinae.

Male courtship behavior is often thought to function to induce the female to allow the male to initiate copulation. If this is true, then male courtship behavior that occurs after copulation has begun ("copulatory courtship") is seemingly functionless and thus paradoxical. It appears, nevertheless, that copulatory courtship is widespread in insects and spiders

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(Eberhard 1991, 1994; Huber in press). It seems likely that copulatory courtship serves to induce other post-intromission female responses that are also critical to male reproductive success, such as allowing the copulation to go to completion, sperm transport (Bukowski & Christenson 1997), dumping the sperm of previous males, storing and maintaining the sperm of the current male, oviposition, and refusing the sexual advances of other males (see Eberhard 1996 for a list of 20 possible female responses).

There is a sizeable, though somewhat scattered, literature on spider mating behavior (reviewed by Robinson 1982; Jackson & Macnab 1991; Richman & Jackson 1992; and Huber in press; for more recent work on araneoids Lubin 1986; Gonzalez 1989; Gonzalez & Armendano 1995; Castro 1995; Bukowski & Christenson 1997). Although there are descriptions of male courtship during copulation (e.g., Jackson & Whitehouse 1989 on the salticid *Thorellia ensifera* [= *Thorelliola ensifera* (Thorell 1877)], in which male tapping appears to induce the female to remain quiet; see also Stratton et al. 1996; and Huber in press summarizing the extensive observations by U. Gerhardt), the emphasis has generally been on pre-copulatory courtship. There are several reasons, however, to suspect that reports have been biased against descriptions of courtship behavior after copulation has begun (Eberhard 1994).

The sexual biology of spiders in the large (> 100 species) genus *Leucauge* White 1841 has been little studied. Both newly molted virgin females and older females mate in the field (Eberhard et al. 1993). Males in the field tend to associate with immature females about to molt to maturity rather than with mature females, suggesting that sperm from a female's first mating are more likely to fertilize her eggs (Eberhard et al. 1993). Castro (1995) described several aspects of the pre-copulatory courtship in *L. venusta* (Walckenaer 1841), *L. "mandibulata"* (the specimens were of *mariana* - H.W. Levi pers. comm.) and the closely related *Plesiometa argyra* (Walckenaer 1841). Brief descriptions of copulatory courtship behavior in *L. mariana* and three other, unidentified species of *Leucauge* were given by Eberhard (1994). Female *L. mariana* build egg sacs on the ground, and cover them with particles of soil and debris (Ibarra et al. 1991). Here we use the very abundant *L. mariana* (Keyserling 1881) to determine the possible significance of male copulation behavior that may be linked to events inside the female during copulation. We also describe the morphological mesh between male and female genitalia, movements of the male genitalia, the process of sperm transfer, and the phylogenetic implications of some aspects of *Leucauge* sexual behavior.

## METHODS

Spiders were observed both in the field and in captivity during September and October 1989 and November 1995 near San Antonio de Escazu, and February–November 1995 in San Pedro de Montes de Oca (both in San José Province), Costa Rica. More than 40 pairs were observed courting and mating in captivity using a 8×, 20×, 40×, and 80× dissecting microscope; verbal accounts of some copulations were taped. Ten copulations were videotaped in captivity at 30 frames per second using a National Newvicon VHS camera equipped with +6 closeup lenses. One mating sequence was videotaped in the field. All drawings depicting the behavior of entire animals were traced from video images. Portions of the spiders that were not resolved in the videos were not drawn. Females whose mating history was known were obtained by collecting penultimate juvenile females that were accompanied by adult males, allowing the females to molt to maturity in captivity (in all cases this occurred within three days or less), and then mating them one to seven days later.

The silk lines on which the spiders met varied, and seemed to have no effect on subsequent courtship and mating. The female was allowed to spin a few lines in an empty wooden or plastic rectangular frame at least 30 cm on a side, or to rest on the orb of another adult female. All males observed in captivity had been collected less than three hours previously; no male was observed more than once. Each pair's behavior was followed until one of the spiders decamped, or until neither had moved for at least 15 min.

The palps of males frozen in liquid $N_2$ during copulation failed to remain coupled to females. The positions of palp structures during hematodochal expansion were therefore determined by squeezing the pedipalp of a copulating male near the base with a pair of forceps during maximal hematodochal expansion, cut-

ting the connection to the male, and then plunging the still inflated pedipalp into Duboscq-Brasil fixative (Romeis 1989). Although the angle of the cymbium with the tibia straightened, the hematodochae remained fully inflated, and the positions of the bulbal sclerites were unchanged after the palp was fixed.

The internal anatomy of male and female genitalia was determined using serial semithin sections (1μm) of specimens fixed in ethyl alcohol or Duboscq-Brasil, then embedded in ERL-4206 epoxy resin and stained with methylene blue in an aqueous borax solution (1%) (see Huber 1993). Voucher specimens have been deposited in the Museum of Comparative Zoology at Harvard University, the American Museum of Natural History, and Museo de Zoología of the Universidad de Costa Rica.

## RESULTS

**Pre-insertion courtship by males.**—The term "courtship" is used here to refer to behavior that was repeated both within and between pairs, that obviously resulted in stimuli being received by the other spider, and that had no obvious mechanical function in bringing and keeping the spiders together (i.e., walking toward the female is excluded). The term "copulation" is used to include all genitalic contact between a particular male-female pair until the male left or became immobile. The term "insertion" designates the entrance of the embolus and conductor into the epigynal opening.

Pre-copulatory courtship was both complex and highly variable, and the descriptions below are only a preliminary list of the different types of behavior. The more complex questions of frequencies and sequences of different behaviors are mostly ignored. The substantial variation in the simple presence or absence of particular types of behavior (e.g., Figs. 3, 8) suggests that frequencies, durations, and sequences of different behavior patterns also may be quite variable. The names correpond, when possible, to names used by Robinson & Robinson (1980) in their review of araneid and nephiline courtship behavior. We have illustrated many behavior patterns due to the difficulty we experienced in comparing our observations with those in previous accounts.

Courtship and copulation occur in nature on intact orbs, and on special molting webs (Eberhard et al. 1993). Of the behavior patterns that males performed before copulation, at least seven may function to stimulate females (all were usually performed while the male was on the same line on which the female was resting):

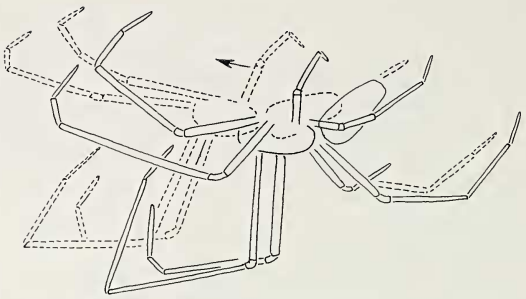


Figure 1.—The forward movement of rocking behavior by a courting male in lateral view (dotted lines follow solid lines by 0.07 sec). The male rocked his body forward and backward by alternately extending and flexing his legs IV.

*1. Jerk:* The male, while facing the female, flexed his anterior legs strongly and quickly (less than 0.1 sec) without releasing the lines they held. The result was a sharp jerk on the web that caused the female's body to swing. These jerks were superficially similar to jerks spiders made in response to prey or other spiders on their webs, and may represent searching behavior rather than courtship. This behavior was similar to that described as "jerk" or "shake" in the courtship of a variety of araneids and nephilines by Robinson & Robinson (1980), and the "jalón" of Castro (1995).

*2. Rocking:* The male flexed and extended his legs IV rhythmically so that his body rocked backward and up, then forward and down (Fig. 1). In several males vigorous bursts of rocking were accompanied by smaller, more rapid flexes that set the male's entire body quivering briefly. Rocking movements were often performed while the male faced away from the female, but also occasionally while he faced her. This behavior may correspond to "vibración o bamboleo" of Castro (1995). The most similar behavior described by Robinson & Robinson (1980) is the "bounce", but this is apparently an up-and-down rather than a forward-and-backward movement as in *L. mariana.*

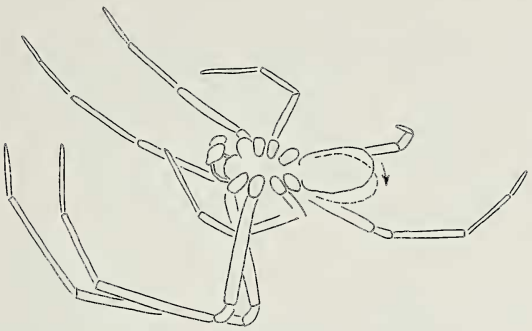


Figure 2.—Abdomen bobbing during pre-copulatory courtship (dotted lines follow solid lines by 0.1 sec) in ventral view. The male's abdomen was repeatedly flicked dorsally briefly.

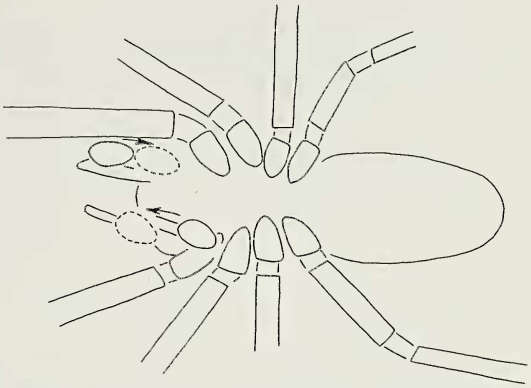


Figure 3.—Ventral view of rapidly alternating palp rubbing movements during pre-copulatory courtship. The posterior movement of the palp (dotted lines on left of drawing, which follow solid lines by 0.07 sec) was followed 0.07 sec later by an anterior movement of the other palp (dotted lines on right follow solid lines there by 0.1 sec).

*3. Abdomen bobbing:* Abdomen bobbing consisted of quick, dorsally directed flexions of the male's abdomen at the pedicel that lasted about 0.07 sec each (Fig. 2). On some occasions it appeared that the abdomen vibrated as it was twitched, while on others a male flicked his abdomen without causing a general vibration of his body, suggesting that these are two different movements. One common context for abdomen bobbing was at the end of a burst of palp vibration (e.g., Fig. 4). Abdomen bobbing occurred both when the male was facing toward and away from the female. Abdomen bobbing was similar and possibly homologous to rapid "abdomen wagging" movements that occur in a variety of araneid spiders (Robinson & Robinson 1980), and the theridiid *Latrodectus* (Ross & Smith 1979).

*4. Palp rubbing:* The male moved his pedipalps in brief bursts of alternate anterodorsoposteroventral movements, with the bulb moving from in front of his chelicerae to just ventral to his endites (Fig. 3). Bursts lasted up to several seconds (Fig. 4), and the palps completed a single rub on the order of about one every 0.2–0.3 sec (Fig. 3); in some cases palp movements became progressively more brisk toward the end of each burst of vibration.

Observations at 20× showed that the palps themselves did not usually touch each other during rubbing; in most cases the bristles on each cymbium probably contacted each other, but this was sometimes not the case. The base of each palpal femur rubbed against the retrolateral surface of the chelicera during palpal rubbing, and in some cases one palp was moved while the other was immobile, supporting the possibility that femur-chelicera contact was an important aspect of these movements. Inspection of a male's cuticle with a compound microscope failed, however, to reveal any special structure where the palpal femur contacted the chelicerae.

Palp rubbing movements were termed "oscilación de palpos" by Castro (1995). They appear to be similar to the "palpal scrabbling" described by Robinson & Robinson (1980), but differ in being performed while the male was not in contact with the female. Palp rubbing movements were much more rapid than those of palp cleaning when the male passed his palps through his mouthparts following copulation.

*5. Twanging:* The male folded his legs III ventrally and strummed the line under which he was walking or hanging repeatedly with alternating lateral movements of the two legs (Fig. 5). Twanging always involved a series of strums, and seemed particularly common at close range, during the final approach to the female prior to cheliceral clasping (Fig. 5). This behavior occurs in many araneids (Gerhardt 1928; Robinson & Robinson 1980; also Blanke 1973, 1986 on *Araneus cucurbitanus* [= *Araneus cucurbitinus* Clerck 1757]; Berry 1987 on *Cyrtophora moluccensis* (Doleschall 1857)). It was noted by Castro (1995) only in *Plesiometa argyra.*

*6. Line tapping:* The male rested under a line leading toward the female, holding it with

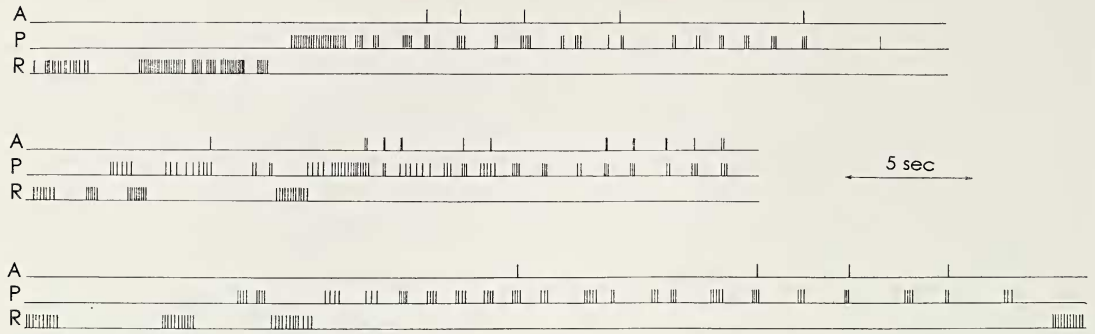



Figure 4.—Patterns of occurrence of abdomen bobbing (A), palp rubbing (P), and rocking (R) in three pre-copulatory courtship sequences in video tapes of one male courting a virgin female. Each vertical line represents a burst of movements. Bursts of rocking and palp rubbing tended to occur in groups. Abdomen bobbing tended to occur in conjunction with palp rubbing, while rocking tended to occur alone.

his partially flexed legs II. Legs I and/or II were held near the line and made quick, mesally directed taps against the line (Fig. 6). The legs apparently did not grasp the line at any time during slapping movements in which the tarsus or metatarsus contacted the line. Usually there were several taps in each series (e.g., Fig. 6). This movement appears not to have been described previously, at least in these terms.

*7. Tapping the female:* The male, especially when interacting with a relatively non-aggressive female, often approached close enough to touch or tap her briefly with his anterior legs, probably with the tarsi or metatarsi. Often after such contact a male turned and moved away several body lengths, then attached his dragline and returned to her along it. Tapping behavior did not seem to be stereotyped with respect to either the parts of the female's body that were contacted or the pattern of movements of the male. This behavior might thus be considered searching or sensory behavior of the male rather than courtship. Nevertheless in some pairs it was the only apparently stimulatory behavior performed by the male be-

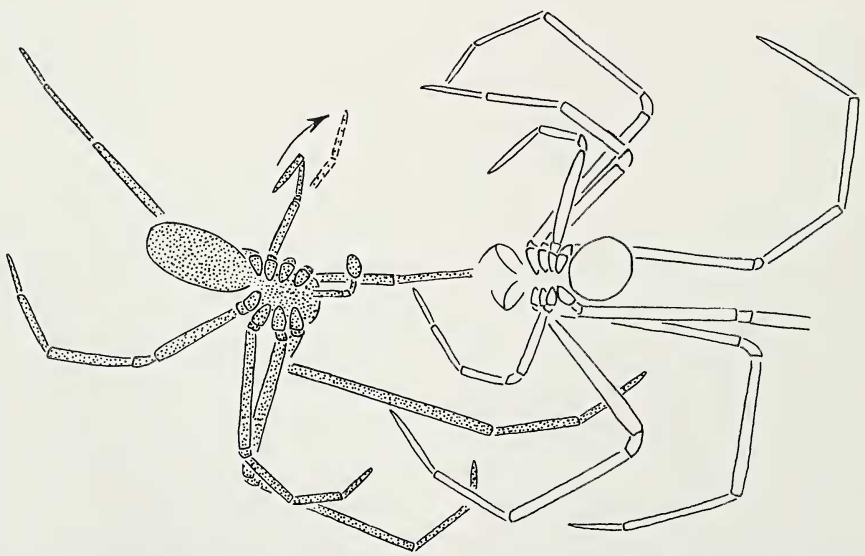


Figure 5.—Twanging with one leg III by a courting male (stippled) seen in ventro-lateral view as he approached a female whose chelicerae were already open to clasp his (dotted lines follow solid lines by 0.07 sec). The male used alternate strokes with his legs III to strum the line along which he was moving toward the female.

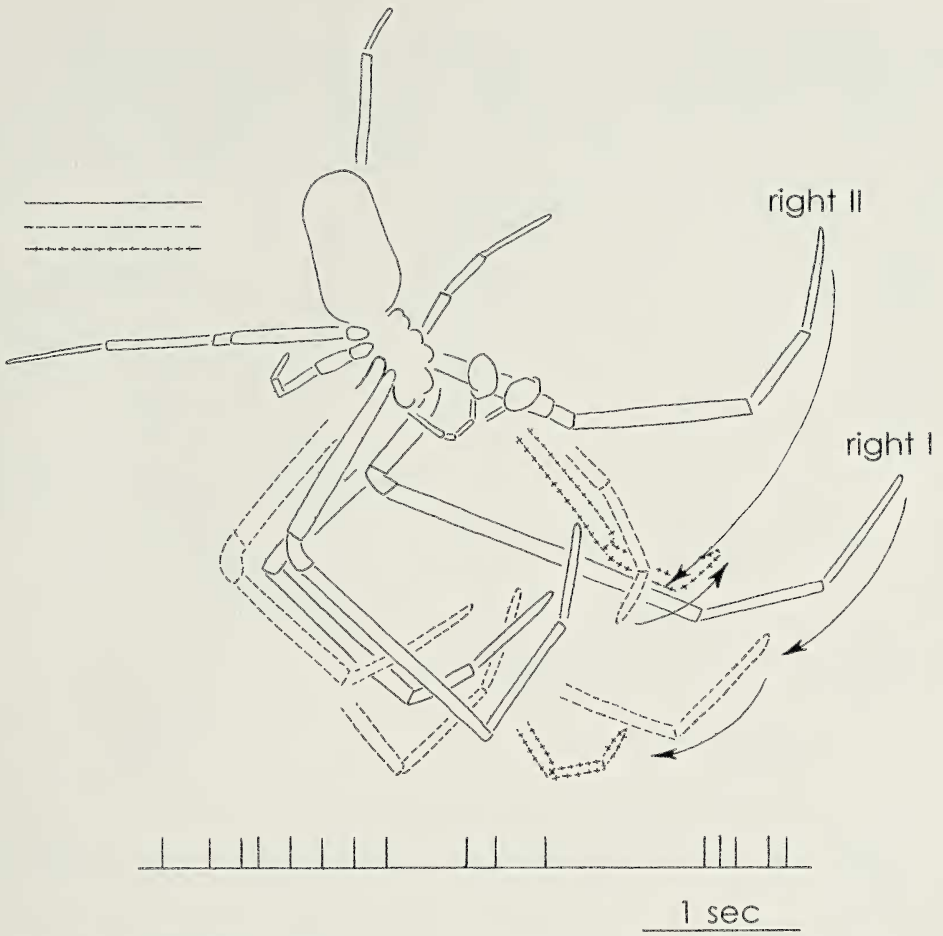



Figure 6.—Line tapping during pre-copulatory courtship by a male seen in ventro-lateral view (dotted lines follow solid lines by 0.1 sec). The male's right leg II moved mesally to apparently tap the line on which he was resting, and immediately moved laterally. His right leg I also moved mesally, hitting the line slightly later than leg II. In the graph each vertical bar is a tapping movement.

fore the female assumed the receptive posture and copulated.

**Female responses.**—We did not attempt to associate particular types of female response with specific male behavior patterns (in most video recordings of male behavior the female was not in view); the general impression was that there was little if any specificity in female responses to particular male signals. Females made three types of responses to male courtship preceding copulation.

*1. Turn toward male:* The female usually turned to face the male when he approached her from the rear, sometimes however only after the male performed repeated bouts of courtship behavior.

*2. Open chelicerae:* The female often repeatedly opened and closed her chelicerae prior to linking with the male (e.g., Fig. 5); presumably these were intention or exploratory movements associated with cheliceral clasping.

*3. Assume mating posture:* Just prior to copulation, the female lowered her body while facing directly toward the male, spreading her anterior legs and opening her chelicerae wide, and often flexing her abdomen ventrally in an acceptance posture (Fig. 5). The female clearly bent her abdomen ventrally in 11 of 12 videotaped pairings in which the angle of viewing was adequate to resolve this detail. In two cases the female later bent her abdomen dorsally while the male was attempting to insert his palp, and in one of these pairs he was un-

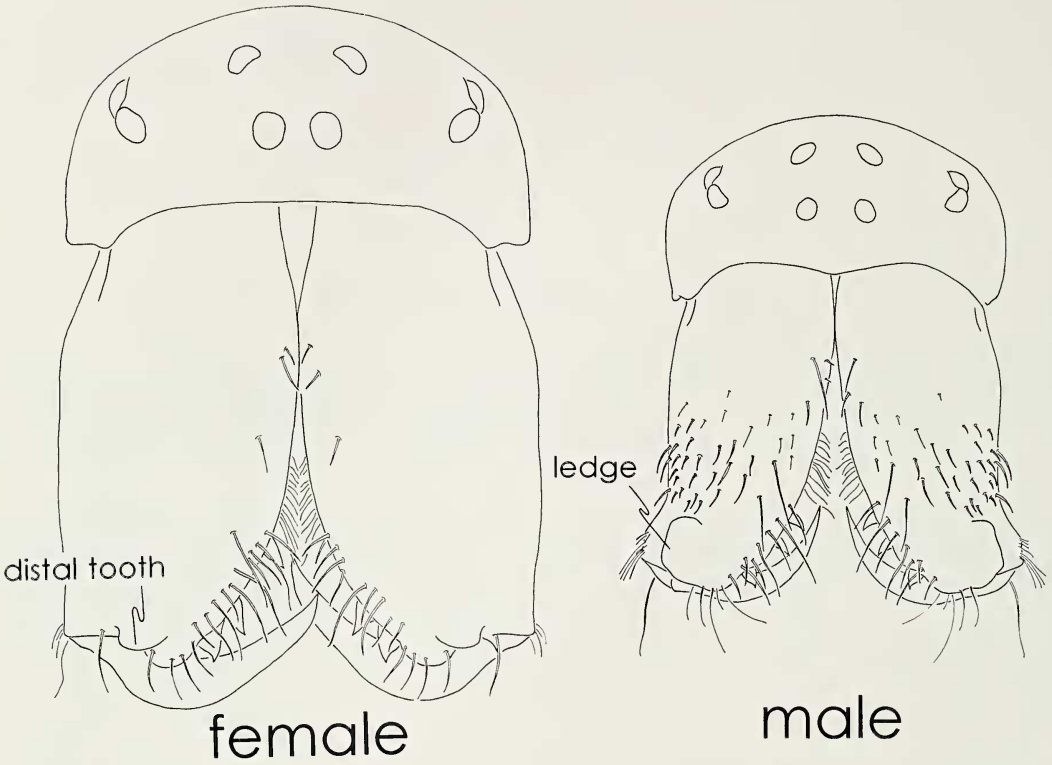



Figure 7.—Frontal view of the chelicerae of male and female *L. mariana* drawn to the same scale. The anterior surface of the basal segment of the male chelicerae has more setae, and a "ledge" that contacts the basal segment of the female chelicerae (perhaps the distal tooth) while she clasps his chelicerae with hers.

able to reach her epigynum as a result. The male often tapped the female with his legs as she lowered herself into position and as she waited there.

**Cheliceral clasp**.—Relatively stereotyped contact involving both the legs and the chelicerae of the male and the female occurred just prior to copulation. The female always opened her chelicerae wide as the male approached (usually with his own chelicerae closed), and then grasped the distal portions of the basal segments of the male's chelicerae by closing her fangs. The inner surface of her fang clearly pressed against the posterior surface of the male's chelicerae rather than against his endites. The modified "ledge" on the anterior surface of each of the male's chelicerae (Fig. 7) was thus pressed against the distal surface of the basal segment of the female's chelicerae. Observations at 8× with a mirror behind the spiders established that the female cheliceral tooth nearest the insertion of her fang was near the ledge on the male chelicerae. Unfortunately the abundant hairs on the border of the female chelicerae made it impossible to see the exact position of the female's tooth with respect to the male's ledge. As the cheliceral clasp was being achieved or just after, the male extended one of his pedipalps to rest on the ventral surface of the female's abdomen.

As the two spiders locked chelicerae, the male positioned his legs I and II so that they were in contact with the ventral surfaces of the corresponding legs of the female and tapped against them. Often his legs III were also held against the legs III of the female, contacting their dorsal surfaces. Usually the male contacted the female with the distal portions of his legs I and II (tarsi, metatarsi).

A given pair of spiders often made several cheliceral clasps during a copulation (Fig. 8, Table 1). Between clasps the spiders moved apart, in some cases several body lengths. The male often courted again before each subsequent cheliceral clasp. In some cases the fe-

Table 1.—Characteristics (averages with one standard deviation) of copulations with virgin females and comparisons with copulations with females that had mated once 1–7 days earlier. Frequencies were compared using Chi Squared Tests; other comparisons were made using Mann-Whitney *U* Tests. Some copulations were observed in more detail than others; this accounts for different sample sizes and missing data. *Significantly different with Mann-Whitney *U* Test, $P < 0.001$.

| | Female Virgin ($n$ = 24) | Female Mated ($n$ = 13) | $P$ |
|---|---|---|---|
| Duration copulation (min) | 17.3 ± 6.1 | 9.9 ± 13.3 | <0.001 |
| Number long insertions | 3.5 ± 2.0 | 0.2 ± 0.6 | <0.001 |
| Number bouts of short insertions | 6.21 ± 5.2 | 4.1 ± 3.7 | 0.201 |
| Number clasps with chelicerae | 2.3 ± 1.2 | 1.7 ± 1.4 | 0.032 |
| Female pushed palp with leg III at least once | 50% | 29% | >0.1 |
| Duration long insertions (sec) | | | |
| first | 109 ± 71 ($n$ = 24) | | |
| second | 123 ± 67 ($n$ = 21) | | |
| third | 121 ± 104 ($n$ = 14) | | |
| Duration of each bout of short insertions (sec) | 40 ± 19 ($n$ = 41 bouts, 7 copulations) | | |
| Number hematodochal expansions during each long insertion | 57.0 ± 26.1* ($n$ = 34 insertions, 7 copulations) | | |
| Number inflations during each bout of short insertions | 14.6 ± 7.0* ($n$ = 41 bouts, 7 copulations) | | |

male's behavior just after a pair broke apart appeared to be aggressive, and she made rapid bursts of movement and gave relatively violent jerks on lines running toward the male. The male nevertheless often courted and successfully induced her to approach again (or allow him to approach), and to assume the acceptance posture. Copulations with virgin females were longer, and included more chelicerai clasps than copulations with non-virgins (Table 1).

**Copulation.**—1. *Leg and abdomen movements:* During copulation males performed at least three behavior patterns seen in pre-insertion courtship: leg tapping with legs I and II, abdomen bobbing, and rocking. During leg tapping the male repeatedly tapped his anterior legs (I, II; sometimes also III) against the female's legs, often on their ventral surfaces (except for legs III). Tapping during copulation differed from pre-copulatory tapping in more consistently involving particular parts of the female's body. Each of the male's legs tapped on the corresponding leg of the female (e.g., male right I on female left I, male right II on female left II, etc.). The order in which legs tapped varied; frequently (but not always) the right and left legs of a pair alternated.

Bursts of tapping usually lasted several seconds (average 4.5 ±1.2 sec, $n$ = 13 bursts by one relatively actively tapping male). Leg tapping occurred during the first moments after the female grasped the male chelicerae and the male attempted to insert his palp, and also nearly always occurred during the withdrawal of one palp and insertion of the other. When, on occasion, there was a pause of a second or more between withdrawal and insertion, leg tapping did not begin until several tenths of a second before the insertion occurred, suggesting that insertion rather than withdrawal is the context for leg tapping. Leg tapping also occurred periodically during long insertions. The rhythm of inflation and deflation of the male's palpal hematodochae was not modified while his legs tapped the female.

Males also performed an additional behavior not seen prior to insertion, bursts of front leg pushing. The male's front four legs were repeatedly extended synchronously against the legs of the female while, in most cases, his legs III and IV were held immobile. Most ex-

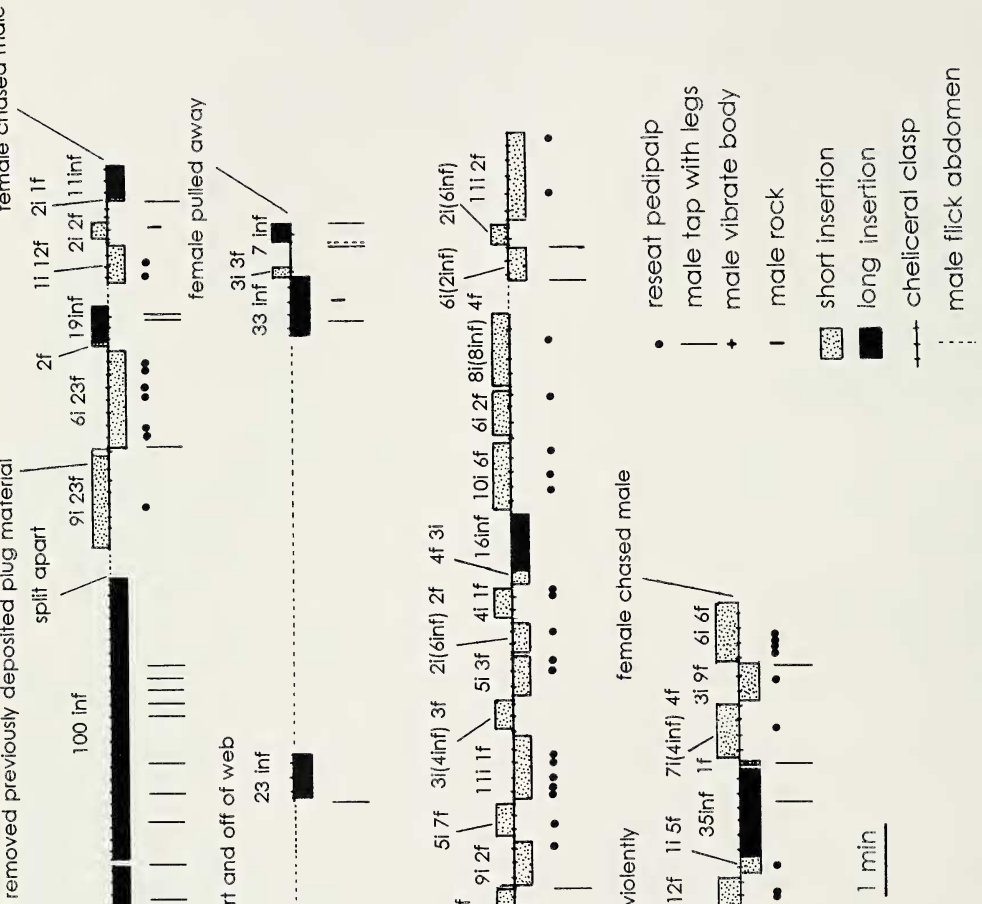



Figure 8.—Graphical representation of the sequence of events in four copulations with virgin females. The blocks representing insertions with the two different palps are accompanied by the numbers of hematodochal inflations (inf) (in the case of long insertions), or of insertions (i) and flubs (f) (in the case of short insertions). Long insertions tended to occur earlier, but there was substantial variation in this and other details of copulation.

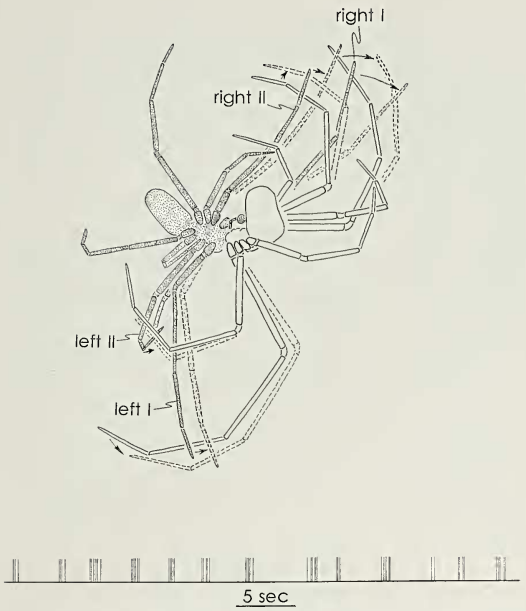



Figure 9.—Rhythmic pushing with the front legs during copulation, seen with male (stippled) in antero-ventral view and the female in postero-lateral view (dotted lines follow solid by 0.07 sec). While the chelicerae were locked, the male's legs I and II contacted the corresponding legs of the female and were extended and moved slightly forward (with respect to the male's body) in synchrony with palpal movements. Legs III and IV of the male were held more or less still (female leg III was out of focus). The graph shows the pattern of pushes by this male (each vertical line is a push). The first push of a series was always stronger than the others.

tension was at the male's femur-patella joint (Fig. 9). Some males pushed only once each time; more commonly, the male made repeated quick series of pushes (Fig. 9). The strength of the pushes varied widely. The number of bursts of pushing during a long insertion ranged from 0–9, and averaged 2.4 for each insertion that had at least one burst of pushing ($n$ = 8 copulations). Bursts of leg pushing began at the same time as the basal hematodocha of the palp was inflated. Deflation, which was much more gradual than inflation, occurred between bursts of pushes.

2. *Movements of the male's genitalia:* Insertion: During each cheliceral clasp, the male extended at least one palp one or more times to contact the female's abdomen. During each extension of a palp the basal hematodocha was expanded one or more times to insert (or attempt to insert) the embolus and conductor into the female's epigynum. Male palps engaged the female epigynum in two different ways—"long" and "short" insertions. Long insertions usually occurred early in a copulation, and short insertions later, but there were numerous exceptions (e.g., Fig. 8). In a long insertion, each palp usually made only a single long insertion before it was withdrawn from the female's abdomen and the other was inserted (Fig. 8) (occasionally these distinctions were not clear, and the conductor and embolus withdrew from the female following each of the first few inflations of the basal hematodocha, and then remained inserted during subsequent inflations—see descriptions of short insertions below, and Fig. 8). In contrast, short insertions occurred in bursts of several short insertion attempts made by the same palp before it was withdrawn and the other palp was extended to the female's abdomen. The duration of a long insertion averaged over a minute (Table 1), while short insertions lasted only on the order of a second or so. As mentioned above, the first insertions in matings with virgin females were usually of the long type (Fig. 8), while copulations with nonvirgins almost never included long insertions (Table 1). The order of long and short insertions was variable (Fig. 8); sometimes a long insertion occurred after several short insertions had been performed on the same side of the epigynum.

Both long and short insertions began in a similar manner. The palp was extended so that the dorsal surface of the cymbium contacted the ventral surface of the female abdomen just anterior to her epigynum. The trochanter projected ventrally, and the distal portion of the tibia passed near the groove between the inner margins of the female coxae IV, but did not touch it (Figs. 9, 13). At least some of the many setae of the cymbium (Fig. 10), especially those in its basal half, were interlaced among the setae near the female's epigynum (Fig. 14). The cymbium was turned and directed somewhat laterally (e.g., the male's right cymbium was directed to his left, so that its distal tip was just to the female's right of her midline). Although it was difficult to make direct observations, it appeared that the long patellar seta (Fig. 10) often (perhaps always) contacted the cymbium on its inner, concave surface; in some cases this seta was displaced laterally as the basal hematodocha was inflat-

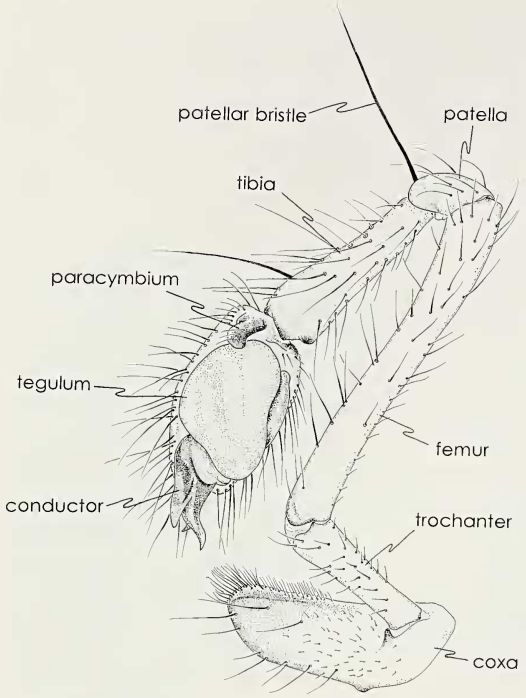



Figure 10.—Entire left palp at rest, showing elongate trochanter, and structures of the bulb (retrolateral view).

ed, confirming that its tip rested on the inner surface of the cymbium.

Inflation: The basal hematodocha was then inflated. The cymbium moved away from the female's ventral surface, and the more distal portions of the palp were displaced away from the cymbium and rotated nearly 180°. During the last portion of this rotation the conductor and embolus moved toward and usually contacted this side of the female's epigynum (i.e., the distal portion of the male's right palp moved to his right, and became inserted into the epigynum on the female's left side). The smaller median hematodocha was inflated during the latter portion of each inflation of the basal hematodocha. It caused the tegulum to move slightly away from the subtegulum, but did not result in any rotation.

In a long insertion, the conductor and embolus, which were driven against the epigynum by the movements produced by hematodochal inflation, remained in contact with the epigynum when the hematodochae partially collapsed. There followed a more-or-less extensive series of approximately simultaneous inflations and collapses of the two hematodochae (Table 1, Fig. 8). During each inflation the distal parts of the palp twisted slightly around the point where the tip of the conductor contacted the entrance of the insemination duct, and the embolus had entered the insemination duct (see below). This movement caused the hook on the conductor process (Fig. 11) to sweep antero-laterally on the female's epigynum until it was arrested just before maximum inflation by hitting the hood on the anterior margin of the atrium (Fig. 14).

During each hematodochal inflation the palp also extended slightly at the femur-patella joint, thus pushing the palp slightly posteriorly on the female's abdomen. The rhythm of expansions was more rapid at the start of a long insertion (avg. 1.1 ±0.34 expansions/sec in the first 20 expansions in the first long insertion of 8 copulations) than later (avg. 0.73 ±0.21 expansions/sec in the last 20 expansions in the same insertions ($P$ = 0.022 with Mann-Whitney $U$ Test).

Positions of bulb sclerites: Hematodochal expansions caused the sclerites of the bulb to change positions relative to each other. All major movements seemed to be caused by the expanding basal hematodocha, while the expansion of the median hematodocha apparently only tightened the contact between bulbal sclerites and the epigynum. During the first hematodochal expansion, the base of the embolus was displaced about halfway toward the tip of the thick portion of the conductor, and rested immobile there with its curved tip meshing with the curved surface of the conductor (Fig. 11). Displacement of the base of the embolus was the result of the tegulum being rotated against the paracymbium (Fig. 12). The paracymbium was lodged in a groove on the tegulum, and the rotation caused it to push against and move the base of the embolus. Once this rotation occurred, the base of the embolus did not move during the rest of a given long insertion. The movement of the embolus was made possible by its membranous articulation with the tegulum.

The tip of the embolus projected 155–165 μm beyond the tip of the conductor in three different males. This distance was nearly the same as the distance travelled by the base of the embolus toward the tip of the conductor (Fig. 11), confirming that the movement of the embolus base caused the embolus to be exerted. The tip of the conductor remained

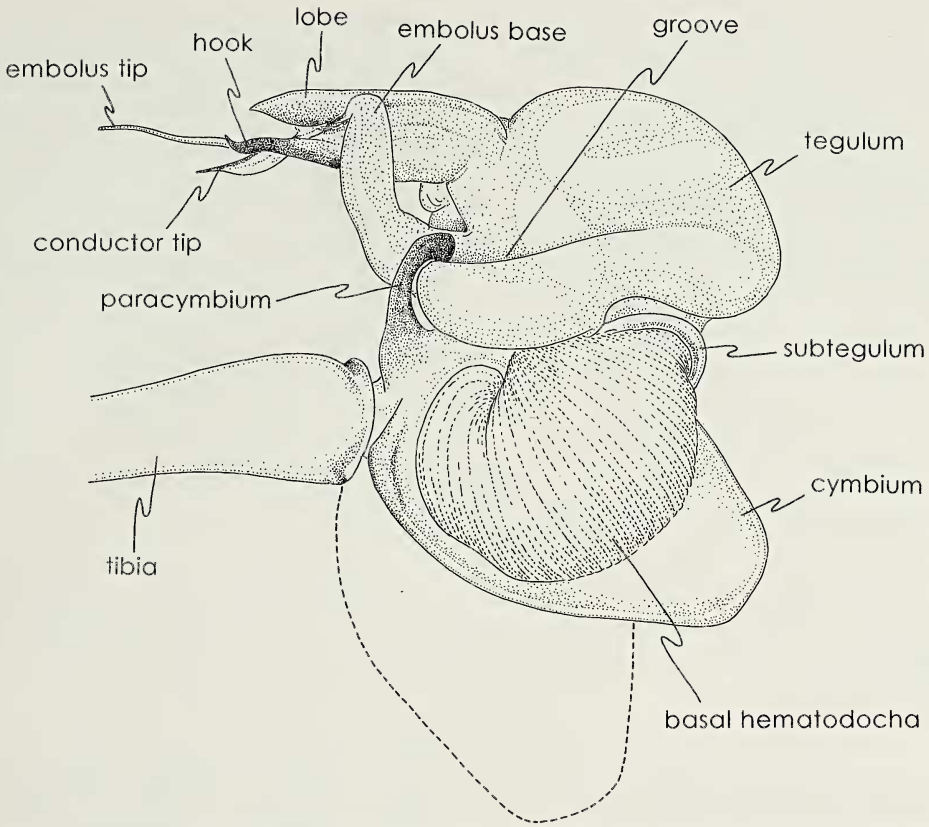



Figure 11.—Ventral view of the distal portion of the right palp with the bulb expanded after being cut from the male and fixed. While the cymbium straightened with respect to its position during insertion (dotted lines) and the hematodochae collapsed (only partially in the case of the basal hematodocha), the distal bulbal structures remained in their natural positions during insertion, with the embolus base erected by the rotation of the tegulum against the paracymbium.

lodged in the entrance of the insemination duct during each long insertion. Thus the tip of the embolus must have passed through the insemination duct and then entered deep into chamber I of the spermatheca (Fig. 15), because the length of the insemination duct of the female was only about 60–80 μm. Since the base of the embolus did not move after the first hematodochal inflation, the embolus presumably remained inserted in chamber I of the spermatheca throughout each long insertion.

After each long insertion, the male withdrew his palp from the female's epigynum. Sometimes he appeared to have difficulty freeing the conductor and embolus, so that only after he had pushed the female with his legs (and sometimes the female had released her cheliceral grip) did his palp come free with a snap.

In the second, short type of insertion, the tips of the conductor and the embolus contacted the epigynum when the basal hematodocha was inflated, as just described, but they rotated back (along with other distal sclerites) to their original position on the cymbium each time the hematodocha collapsed. Usually the same palp was inflated repeatedly before being withdrawn; the number of insertions averaged about five (Table 1, Fig. 8). A burst of short insertions lasted on average less than half as long as a long insertion, and included only about one fourth as many hematodochal inflations (Table 1, Fig. 8). Each time the palpal sclerites rotated to bring the tips of the conductor and the embolus into contact with the epigynum, the base of the embolus was gradually forced toward the tip of the conductor by the paracymbium as in long insertions. The maximum displacement of the base of the

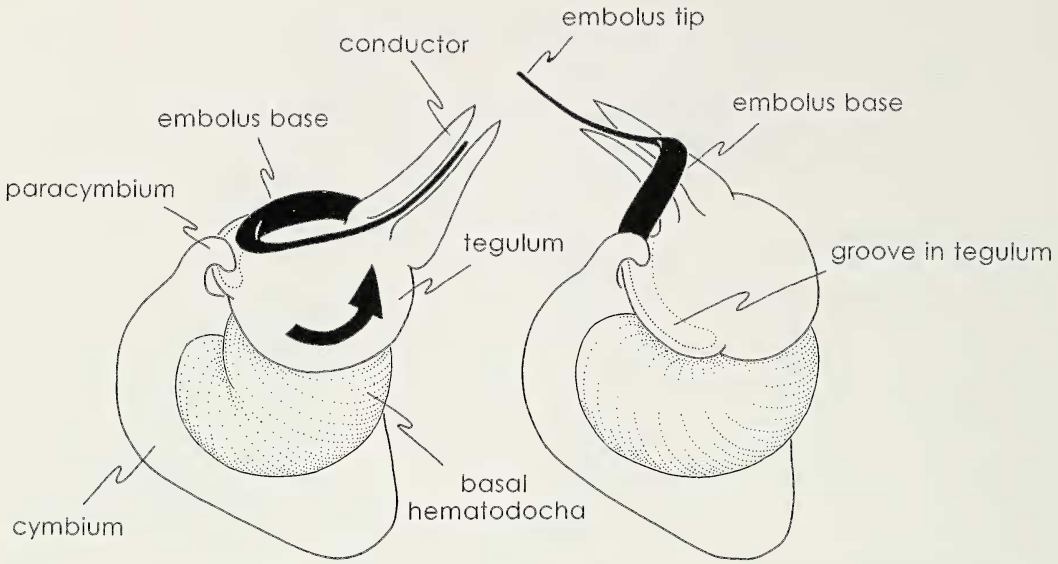



Figure 12.—Schematic representation of movements of palpal sclerites that result in projection of the tip of the embolus. The rotation of the tegulum (arrow at left) causes the paracymbium, which is engaged in a groove on the tegulum, to push against the embolus base, and this causes the embolus tip to emerge from the conductor.

embolus was sometimes about the same as that during a long insertion (Fig. 11), but often it moved only part of the way along the conductor. In contrast to long insertions, the base of the embolus returned to its position alongside the tegulum each time the hematodochae collapsed. The median hematodocha also inflated during each inflation cycle of a short insertion, but it was partly hidden; and it was thus not possible to determine exactly when its inflation began with respect to movement of the base of the embolus. It was clear, however, that inflation of this hematodocha continued slightly after the base of the embolus had stopped moving distally.

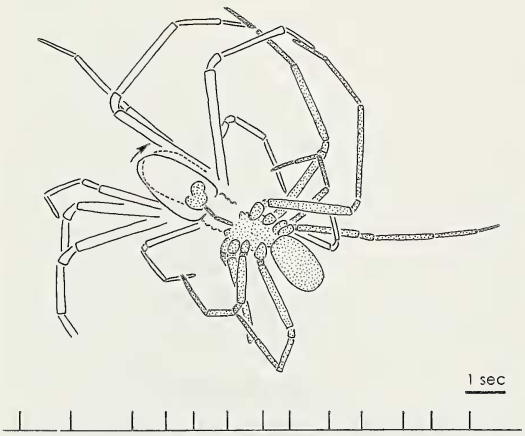



Figure 13.—A short insertion of the right palp of a male (stippled) (in postero-ventral view) caused the female's entire abdomen to be displaced (dotted lines follow solid lines by 0.07 sec). The graph below shows the rhythm of insertions as revealed by displacements of this female's abdomen (each vertical line is a displacement).

In some pairs the conductor and embolus pushed so forcefully on the female during each inflation that her abdomen was twisted or deflected perceptibly each time the basal hematodocha inflated (Fig. 13). Judging by these twists in video recordings, the time taken to inflate the hematodocha in one pair was 0.07–0.1 sec; after 0.2 to 0.3 sec, the abdomen gradually sagged back to its original position, remained there for about 0.1 sec more until it was twisted again (Fig. 13).

Flubs: A third type of palpal contact represented apparent failed attempts at insertion ("flubs" in the terminology of Watson 1991). Inflation of the hematodochae caused the tips of the conductor and the embolus to scrape across the face of the epigynum without engaging it as in a successful insertion, or briefly engaging it at an inappropriate site. In one pair, for example, the conductor engaged and was briefly inserted into the slit (Fig. 14) of

the opposite side of the epigynum several times. Flubs were common during bouts of short insertions. In seven copulations (involving 41 bouts of short insertions), there was an average of 8.7 ±4.9 flubs, and 6.6 ±3.7 successful insertions in each bout.

The male often lifted his cymbium from the female's abdomen and then set it down at a slightly different site while making insertion attempts (Fig. 8). Repositioning was more likely to occur following a flub. For instance, 79.6% of 54 repositionings in four copulations occurred following a flub, but only 59% of the 337 insertion attempts in these copulations were flubs ($\chi^2 = 9.46$, $P < 0.01$). Flubs were more common later in a copulation, when short insertions occurred (Fig. 8). The number of flubs varied widely; in eight copulations with virgins the frequency averaged 44%, and ranged from 0% (of 9 insertion attempts) to 73% (of 87 attempts).

3. *Transfer of material to the female and subsequent events:* Sperm were introduced into the large, soft-walled chamber I of the spermatheca (Figs. 14–16) during long insertions, causing it to inflate (compare Figs. 15, 16). The total volume of chamber I of one spermatheca when it was inflated was about 6 × $10^6$ $\mu m^3$. In one pair killed and sectioned immediately after a single long insertion, one spermatheca had a mass of sperm (all encapsulated), and the other was still collapsed. The sperm duct of the palp that had been inserted (estimated volume was about 9–11 × $10^6$ $\mu m^3$) was about 70% full. The bulb of another male fixed and sectioned just after a complete copulation was almost completely empty.

The dorsal portion of the wall of chamber I of the spermatheca had an array of small pores that were the openings of glands associated with the wall (Fig. 17). In a female fixed 21 min after the end of a long copulation and then sectioned, a dark-staining fluid similar to that in the cells of these glands was present in chamber I near these pores (Fig. 18). Sperm in the portion of chamber I near the pores in the wall had become decapsulated (Fig. 18). The encapsulated sperm in chamber I were accompanied by much less other material than they had been while in the sperm droplet (Fig. 20) or while in the male pedipalp. In two additional females fixed later after copulation (one collected in the field, the other two days after a single copulation) there

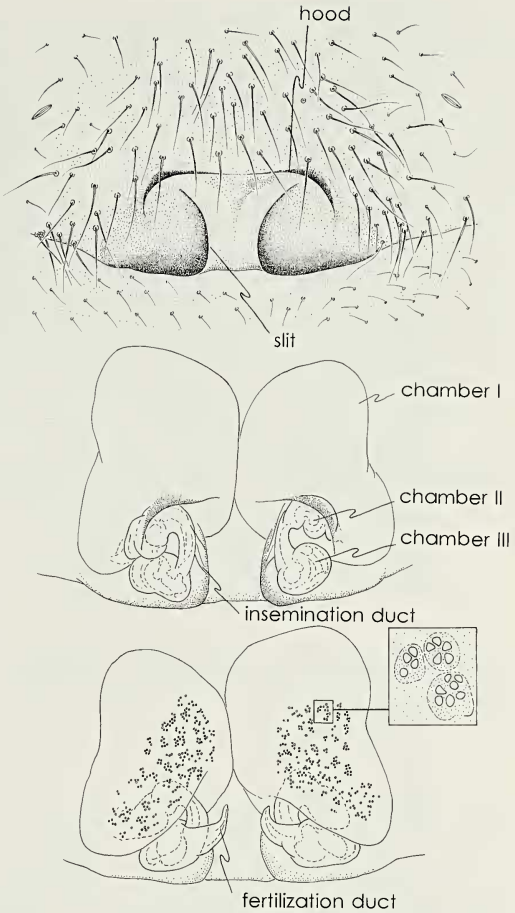



Figure 14.—Top: Female epigynum in ventral view. Middle: vulva of a mated female (cleared in KOH) in ventral view. Bottom: vulva of a mated female (cleared in KOH) in dorsal view (inset shows gland pores in wall of chamber I).

were both encapsulated and decapsulated sperm in chamber I of the spermathecae, and chambers II and III were more tightly packed with decapsulated sperm in small amounts of fluid (Fig. 19). Another female fixed two days after a single copulation also had an additional mass of decapsulated sperm in a small expanded portion of the uterus where the two fertilization ducts emptied. Decapsulated sperm allowed to dry on a glass slide had tails about 17.4 μm long, and curved heads about 7.9 μm long.

During most inflations during short insertions, a viscous white material with an apparent consistency similar to that of toothpaste emerged from the tip of the palp (since no other openings were observed in sections of

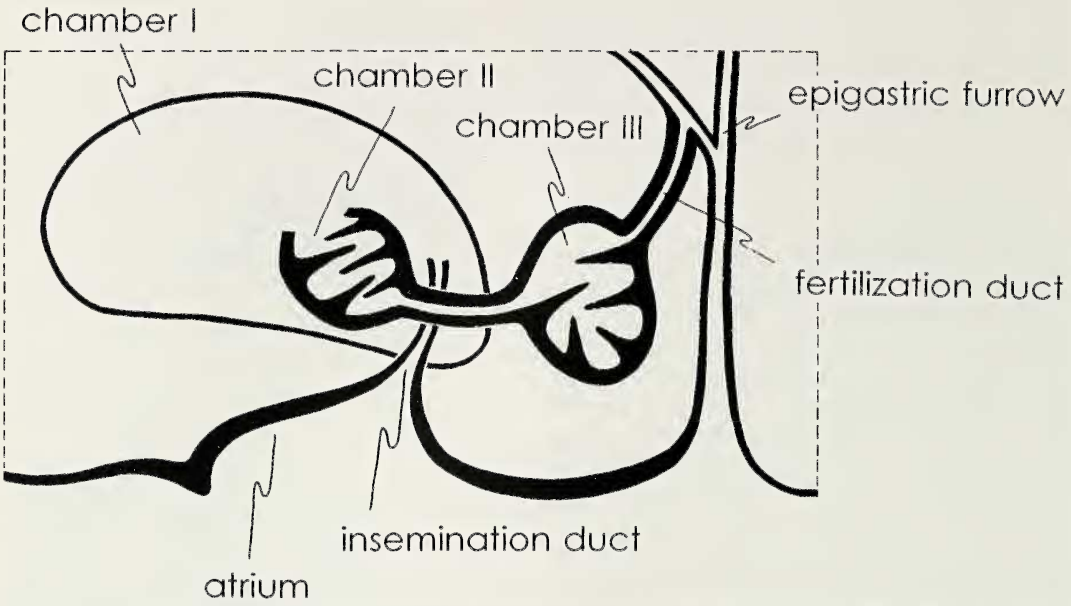


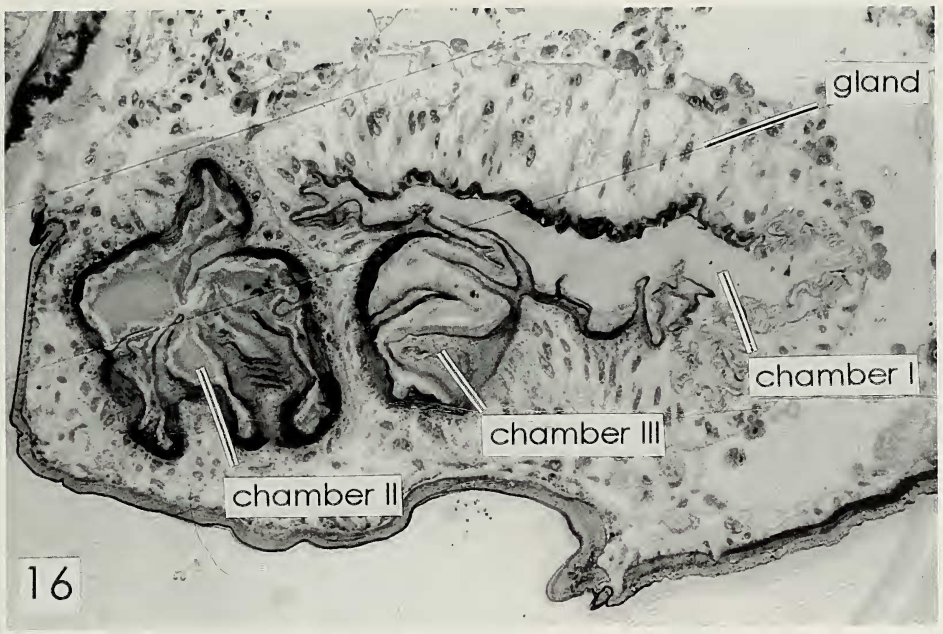

Figure 15.—Schematic lateral view of the internal genitalia of a female (anterior side to the left, ventral side at the bottom). The thin-walled chamber I of the spermatheca, which is collapsed in virgin females, is drawn in its expanded state when filled with sperm and other material (see Fig. 16). Decapsulated sperm occurred in both chamber II and III, as well as in the uterus.

palps, this material presumably emerged from the tip of the embolus). The material emerged while the base of the embolus was being moved by the paracymbium. Since this white material often remained on the outer surface of the epigynum after a copulation was complete, it may be designed to serve as a copulatory plug (or a component of a plug - see below).

In most cases, however, the white material adhered only very poorly to the female. Sometimes it came away still stuck to the male's palp when the embolus and conductor were withdrawn. Often when the tip of the conductor and the embolus were reinserted they dislodged a mass of material that had been deposited previously. During one copulation, for instance, the male more or less filled one side of the atrium with white material three different times, but each time dislodged the accumulation as a result of subsequent insertions. Most copulations with virgin females ended with the female still lacking a plug, even though the male had apparently attempted to deposit one.

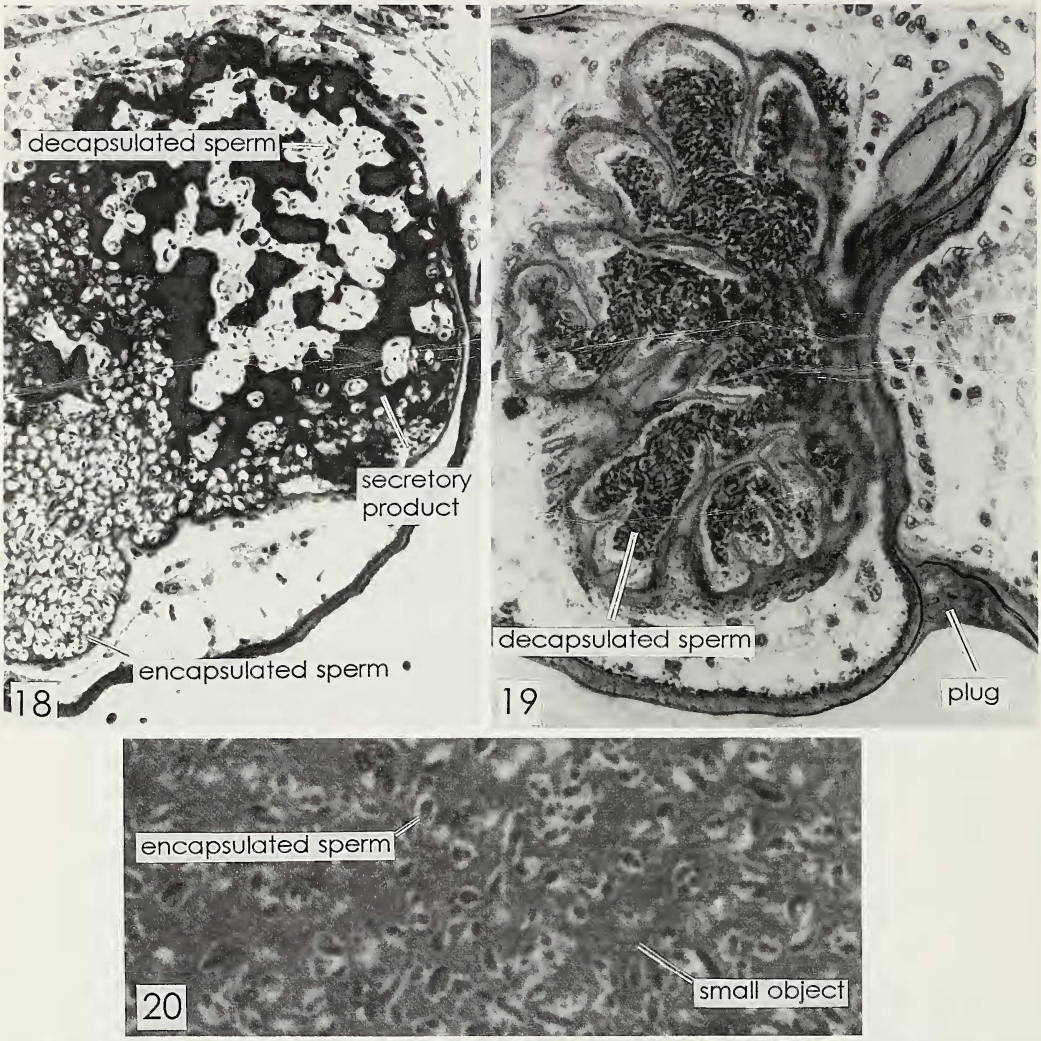
In two cases the plug material assumed a more liquid consistency, and flowed into the atrium and presumably at least into the mouths of the insemination canals, where it condensed into a single, smooth mass that remained in place at the end of copulation. Careful observation of an additional female showed that a similarly liquid plug material had acquired a clearer, less-white appearance and was still very liquid in appearance an hour after copulation ended. The prominent pile of material that had accumulated while the male deposited it had sunk, and had acquired a more level, smooth surface. Similar smooth-surfaced masses were found in the epigyna of many field-collected mature females. Another indication that the material at least sometimes apparently remained liquid in consistency for several minutes was that in some pairs the male performed a long deep insertion on the same side on which he had already deposited material during a shallow insertion.

We had the impression that in the cases in which the female did have a smooth-surfaced plug following copulation, that liquid had emerged from within the female's insemination duct during copulation and combined with the material from the male's palp. When a plug was pulled off the epigynum of a field-collected female, liquid quickly welled up from the insemination ducts and formed a





Figures 16, 17.—Internal structure of *Leucauge* abdomen. 16, Saggital section of the abdomen of a virgin female (anterior side to right, ventral side at bottom), showing the collapsed chamber I and its associated gland, and the complex walls of chambers II and III; 17, Section of the dorsal wall of chamber I of the spermatheca, showing glandular cells with a secretory product apparently being transferred to the lumen of the chamber.




Figures 18–20.—Sperm of *Leucauge*. 18, Saggital section of chamber I of a female shortly after copulation. Sperm in the lower portion of the chamber are still encapsulated and highly concentrated, while sperm in the upper portion, where there is an abundance of a dark-staining material similar to that seen in the gland cells associated with the wall of chamber I, are dispersed and decapsulated; 19, Section of chamber III of the spermatheca of a singly-mated female tightly packed with decapsulated sperm. Plug material (containing encapsulated sperm) is on the surface of the epigynum; 20, The components of a sperm droplet, encapsulated sperm, small round bodies - and a matrix, as seen in a droplet taken from a male's sperm web.

golden crust (as did the liquid which emerged from a puncture wound on the leg). Once the crust formed, the liquid below was withdrawn back into the female's body. Addition of liquid to the male's plug would explain why epigyneal plugs in females collected in the field consistently had smooth outer surfaces and more-or-less filled the atrium of the epigynum, while the material seen being deposited by males during most copulations was in small irregularly-shaped masses of highly viscous material. Of four plugs examined in sections, one clearly contained encapsulated sperm embedded in a matrix, two contained unidentified granules, and one consisted of clear matrix only.

**Sperm induction**.—Transfer of sperm from the male's gonopore to his palps was observed under a dissecting microscope with four different males 15–60 min after copulation. After

building a "Y" shaped sperm web, the male climbed on top of it and made a small central triangular sheet of fine silk, repeatedly bobbing up and down and apparently drawing silk from his epiandrous glands. Both his legs III were extended ventrally, contacting the sides of the triangle near the bases of their femora. The male then deposited a drop of pearly white liquid at the posterior edge of the sheet (near the base of the isoceles triangle), immediately moved under the sheet, and began taking up this liquid by inserting the tips of his palps (the tips of the embolus and conductor) into it in strict alternation. In three cases the male dipped his palps into the droplet 17–20 times in 30 sec near the start of induction; near the end of the 2–5 min process, the rate of dipping had slowed to 8–15/30 sec. One droplet measured 350μm in diameter, giving an estimated volume of $22.4 \times 10^6$ $\mu m^3$. The estimated volume of a single palpal sperm duct, calculated from sections, was approximately $9.5–10.5 \times 10^6$ $\mu m^3$. Thus the sperm droplet probably completely filled the sperm ducts of both palps.

Each immersion lasted only about a second. The tip of the palp touched the anterior surface of the droplet, and then sometimes jerked slightly once or twice as if tapping the droplet. There was an immediate flow of material onto the tip of the palp when the palp first touched the droplet. The liquid appeared to be relatively viscous, and when the tip was pulled away, the surface of the droplet was briefly pulled into a small cone. A small sheath of liquid remained on the tip of the conductor when it was pulled away; there was no perceptible reduction in the amount of this material during the period while the other palp was inserted into the droplet. There were no discernable movements within the palp, or of any of the palpal sclerites at any time during sperm induction.

When the droplet of liquid had almost disappeared, the probing movements of the palps became more insistent, as if the spider sensed that it was sometimes failing to contact the liquid. This impression was reinforced by an inadvertent "experiment". Each time one male pulled his left palp away from a particularly scanty sperm web, nearly the entire sperm droplet adhered to the tip of the palp. Thus every time the right palp was brought into position to take up sperm, there were only tiny droplets left on the sperm web. The right palp of this male clearly probed more actively than did the left throughout sperm induction.

When a recently deposited droplet was examined under a compound microscope and in serial sections, it proved to consist of a liquid matrix containing many small round objects and a smaller number of larger, oval encapsulated sperm (Fig. 20). The sperm ducts in sections of filled male palps also contained encapsulated sperm and smaller granules embedded in a similar matrix. The basal portions of the sperm duct contained the clear homogeneous matrix that filled the entire sperm duct in "empty" male palps before sperm induction.

**Failures.**—Not all pairings resulted in long palpal insertions; some insertions appeared to be interrupted by the female before the male was finished, and some hematodochal inflations failed to engage the conductor and embolus of the palp in the opening of the female's insemination duct ("flubs"). The apparent reasons for these failures are of special interest, as they suggest the types of "problems" that mating males are under selection to solve.

Probably the most common problem, which was mentioned above, was that the "plug" material did not adhere to the female's epigynum. It was not certain whether this problem was due to the male or to the female, though our incomplete observations suggest that female failure to emit liquid from her insemination ducts was involved.

Another common problem resulted from active female rejection behavior. The female used one of her legs III to kick or push the male's palp away from her epigynum (as also occurs in *Nephila* Leach 1815 and *Cyrtophora* Simon 1864 (Gerhardt 1933; Blanke 1972), or held the palp with the tip of one leg III and flipped her abdomen dorsally, jerking the palp free from the epigynum (Fig. 21). This type of rejection was common (44% of 36 copulations), and usually occurred during short rather than long insertions. It was not significantly more common in copulations with virgin females than with non-virgins (Table 1). In some cases the male succeeded in at least temporarily blocking or in displacing the female's leg III with one of his own legs III, preventing her from contacting his palp. Con-

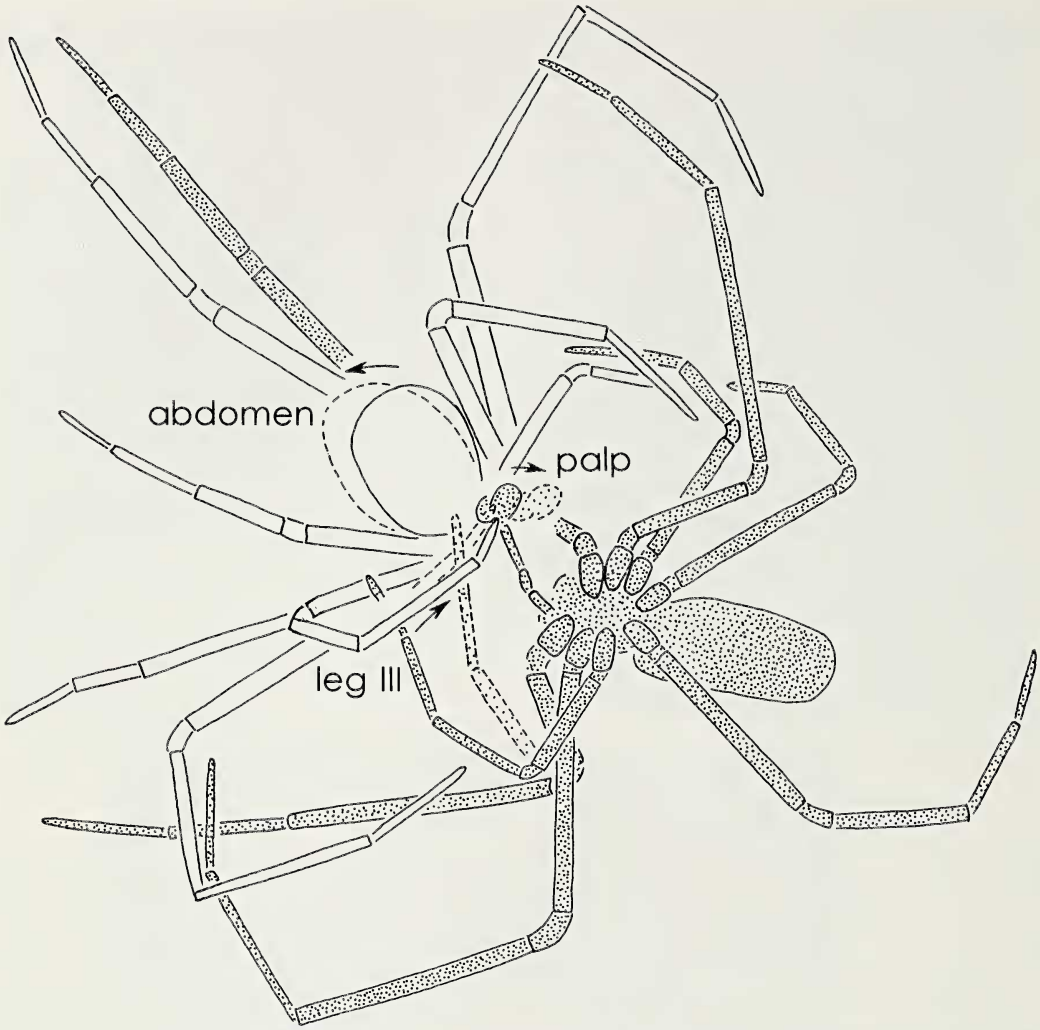



Figure 21.—A female (seen in posterior view) dislodges the palp of a male (stippled) from her epigynum. Although the male moved his leg III to block her (dotted lines follow solid lines by 1.03 sec), the female pushed his palp with her tarsus III (dotted lines follow solid lines by 0.03 sec), and then quickly flexed her abdomen dorsally (dotted lines follow solid lines by 0.03 sec).

certed kicking attempts by females invariably dislodged the palp, however.

Still another problem occurred when the female released her grip on the male's chelicerae and the pair sagged apart while the male attempted to insert his palp. On several occasions the female released the male's chelicerae during a long insertion; in these cases the male simply continued his cycle of hematodochal inflation and deflation, and in two cases the female then resumed her grip while the male continued the long insertion. But in other cases, when the male's palp was not anchored in the female, the separation that occurred when the female released her clasp apparently made it difficult for the male to align his palp properly on her ventral surface and achieve insertion. On one occasion the male responded to being released this way by repeatedly opening and closing his own chelicerae, pressing on the female's fang as he did so. This female eventually opened her chelicerae and the male thrust his own chelicerae between them, thus initiating another cheliceral grasp.

Another possible problem involved the apparent association between flubs and incomplete ventral flexion of the abdomen (see fe-

male acceptance posture above). In one case a relatively small male succeeded in making one insertion, but failed in many subsequent attempts when his palps failed to reach the female's epigynum, and he finally abandoned her. Apparently this male's difficulties were a result of the female failing to bend her abdomen far enough toward him. In several other cases the female appeared to cause flubs, when she deflected her abdomen dorsally during an apparent attempt to make a short insertion.

Overt female aggression toward the male was rare, and did not appear to be a problem, at least during copulation. In one case a female began wrapping the male with silk during a long insertion; the male continued inflating and deflating his hematodochae as before, and escaped readily when the cheliceral clasp ended.

## DISCUSSION

**Courtship before and during copulation.**—The function of male courtship behavior is generally presumed to be to stimulate the female in ways that increase the male's chances of fertilizing her eggs. Demonstration of such a function is not easy, and definitive proof must rely on experimental manipulations of stimuli received by the females. Such manipulations have not been performed with *L. mariana* (nor, indeed, with the large majority of species in which courtship has been studied—see Andersson 1994), so it is necessary to use indirect indicators of probable courtship function. The criteria used here were the following: a) the male's behavior is likely to have caused stimulation of the female; b) the male's behavior was apparently irrelevant to the mechanical problems he experienced in achieving and maintaining genitalic coupling; and c) the male's behavior was repeated during given copulations and in different pairs in relatively stereotyped form. With these criteria, between five and seven types of pre-insertion courtship movements and four types of non-genitalic copulatory courtship occur in *L. mariana.* Copulatory courtship also occurs in *L. venusta* (Walckenaer 1841) (see Castro 1995), and three other unidentified species of *Leucauge* White 1841 (Eberhard 1994), and differs qualitatively among these species (Eberhard 1994).

An additional possible source of stimulation by the male is the substantial displacement of the female abdomen during some palpal insertions (e.g., Fig. 12) (see Coyle & O'Shields 1990 for similar rhythmic movements in the diplurid *Thelochoris karschi* Bösenberg & Lenz 1894, and Huber & Eberhard 1997 on the pholcid *Physocyclus globosus* (Taczanowski 1873)). The number of hematodochal inflations appears to vary dramatically between species, as is expected to often occur in courtship movements under sexual selection (West-Eberhard 1984). Castro (1995) observed an average of over five times more inflations in *L. mariana* (average 103 for 27 copulations) than in *L. venusta* (average 20.8 in 26 copulations) (all copulations were with virgin females). There may also be intraspecific variation in the numbers of inflations, as the average for eight copulations of *L. mariana* in this study was 219 ±71, more than double the average seen in the Mexican population studied by Castro (it is also possible, though seemingly improbable, that the criteria for inflations were not the same in the two studies).

It is not certain whether non-genitalic copulatory courtship in *Leucauge* is an unusual phenomenon among spiders, as might be suggested by the general lack of descriptions of similar behavior in the reviews of courtship and copulation in araneids (Robinson & Robinson 1980) and other spiders (Robinson 1982). There are some reported possible cases of copulatory courtship. For instance, repeated male leg extensions occur during mating in *Nephila* and *Gasteracantha* Sundevall 1833 species (Robinson & Robinson 1980); male abdomen "pumping" occurs in the theriid *Achaearanea wau* Levi, Lubin & Robinson 1982 (Lubin 1986); and rhythmic male abdomen vibrations occur in the pholcid *Physocyclus globosus* (Eberhard 1994; Huber & Eberhard 1997). Apparent non-genitalic copulatory courtship movements by males are known in several species of lycosids (Rovner 1972 on *Lycosa;* G. Stratton pers. comm. on *Hogna* Simon 1885 and *Rabidosa* Roewer 1960). Huber (in press) noted descriptions of apparent copulatory courtship in 31% of 151 species whose behavior was studied by U. Gerhardt.

Underestimates of copulatory courtship are certainly feasible, and in fact one of us (WGE) had previously failed to notice male courtship

movements during observations of several *L. mariana* copulations until after having developed a theoretical reason to suspect that copulatory courtship might occur. Leg tapping is easily misinterpreted as attempts by the male to reposition his legs, until it is noted that the movements occur only in certain contexts, and that the female's legs are usually immobile and not shifting so as to require repositioning by the male. Pushing movements at first seem to be inadvertent extensions of the male's legs associated with changes in internal hemolymph pressure during hematodochal expansions, until it is noted that the third and fourth legs are held completely still, and that some males do not perform pushes while rhythmically inflating their hematodochae. It is sobering to see in one's own observations the strong influence of theory on supposedly objective gathering of empirical data.

An additional aspect of spider mating behavior that may have courtship effects is the often repeated genitalic contact (Huber in press on observations of U. Gerhardt) (for evidence that repeated genitalic contacts can have such a function in other animals, see Eberhard 1996). Patterns of male-female contacts leading to insertions (e.g., cheliceral clasps), of insertions themselves, and of hematodochal expansions during insertions are often complex and variable in different groups of spiders (e.g., Costa & Sotelo 1986 and Stratton et al. 1996 on lycosids; Peaslee & Peck 1983 on an uloborid). The behavior of *L. mariana* was complex in all three respects (Fig. 8).

Secondary sexual modifications of the morphology of male *L. mariana* include a cheliceral process that may be grasped by the female's chelicerae ("ledge" in Fig. 7), and more abundant, stiff setae on the anterior surface of the chelicerae. Similar modifications of the male chelicerae occur in other species of *Leucauge* and in the closely related *Plesiometa argyra* (Castro 1995; W. Eberhard unpubl.). The clasping behavior reported here and by Castro (1995), and the differences among these species suggest that these cheliceral modifications (especially the ledge) may constitute non-genitalic contact courtship devices (Eberhard 1985). It is also possible that they are used as threat devices in male-male battles (especially the setae), as the chelicerae of male *L. mariana* and *P. argyra* may make contact during intense fights between males (W. Eberhard unpubl.).

Non-virgin females, at least when of the ages used in the present study, were clearly more aggressive toward males, and some pre-insertion courtship may function to reduce the female's aggression. Females are, however, apparently not especially dangerous for males, as no males were killed and one male easily escaped after the female wrapped him with silk. Castro (1995) observed females killing copulating males in captivity, however, in *L. mariana* (4 of 48 copulations), *L. venusta* (1 of 72), and *P. argyra* (3 of 26). Alvarez (1992) also saw a female *P. argyra* kill a copulating male.

This study has documented several processes that females perform during or soon after copulation that could affect a male's chances of fertilizing her eggs. There are several female behavior patterns necessary to just permit a given *L. mariana* copulation to proceed successfully to termination: not kick the palp away from the epigynum; bend the abdomen ventrally so the male palp can reach the epigynum; maintain the cheliceral clasp; and remain immobile rather than walk away or attack the male. Females can and sometimes do interrupt copulations in all of these ways. They may also affect male attempts to plug their epigyna by adding or not adding liquid to the male's plugging material, and could conceivably affect the decapsulation of a male's sperm by varying the amount of glandular product added to chamber I of the spermatheca. There are also several post-copulatory female processes, such as oviposition, and rejection of additional mating attempts (Eberhard 1996) that we did not study and that could affect a male's reproductive success. There is thus an ample range of female responses that male copulatory courtship behavior may serve to induce in *Leucauge.*

The trend for more long palpal insertions to occur in copulations with virgin females resembles copulatory patterns in the theridiid *Achaearanea wau* (see Lubin 1986), and the salticid *Phidippus johnsoni* [= *Dendryphantes johnsoni* (Peckham 1883)] (see Jackson 1980). Entelegyne spiders often show first male sperm precedence (Austad 1984; Christenson 1990; see however Masumoto 1993; Eberhard et al. 1993), and these differences in copulatory behavior may be associated with

sperm precedence patterns. It is not clear, however, why fewer and shorter insertions are more appropriate for matings with non-virgin females. In some spiders very short copulations are just as effective in transferring sperm as much longer copulations (e.g., Jackson & Hallas 1986 on the salticid *Portia* Karsch 1878).

The variation in male courtship behavior both before and during copulation was striking, and resembles similar variability in precopulatory courtship in many other orb weavers (Robinson & Robinson 1980). In general, this variability argues against the idea that male courtship functions to inform the female of his species identity. The fact that cross-specific pairing of *L. mariana* and *L. venusta* did not result in initiation of clear pre-copulatory courtship, much less in male-female contact or copulation attempts (Castro 1995), also argues against a species isolating function, especially for copulatory courtship behavior. A more likely explanation, especially for copulatory courtship, is sexual selection (Eberhard 1996), or male attempts to inhibit female aggressive behavior following copulation. Perhaps variety or unpredictability *per se* is stimulatory to the female (e.g., West-Eberhard 1984; Eberhard 1985).

**Genital mechanics, sperm transfer, and plugs.**—It has been proposed that the relative simplicity of tetragnathid palpal morphology, as compared with the complex morphological features that serve locking and bracing functions in the palps of other araneoid spiders during copulation (e.g., Grasshoff 1973; Huber 1993, 1995), is a mechanical correlate of cheliceral locking between male and female during copulation (Levi 1981; Kraus 1984). The cheliceral clasp is thought to give the male solid purchase on the female's body, eliminating the need for palpal locking mechanisms. Several details of *L. mariana* matings argue against this interpretation: 1) The female rather than the male performs cheliceral clasping. This means that the male is not in control of his supposed purchase on the female. If the female's grasp on the male is sufficiently unreliable (and female *L. mariana* often release males during copulation), then it will not be advantageous for the male to eliminate locking structures on his palps. 2) During insertion the tip of the male's palp is far from the point of cheliceral contact, and his relatively long palpal trochanter, femur and tibia are not braced in any way by the locked chelicerae or the female's body. In fact, cheliceral clasping results in the base of the male's palp being *farther* from the female's epigynum than in many other araneids. The relatively long distance between the basal segments of the male's palps and the epigynum also means the female must bend her abdomen ventrally to allow copulation, making the male's coupling with the palp even more precarious (an even stronger ventral flexion of the female abdomen must occur to allow copulation in some species of *Tetragnatha* Latreille 1804 and *Pachygnatha* Sundevall 1823 (Gerhardt 1921, 1928; Kaston 1948; Levi 1981; W. Eberhard unpubl.). Sometimes a female *L. mariana* did not bend her abdomen enough for a male to reach her epigynum. 3) The sclerites of the palp of *L. mariana* roll free on the ventral surface of the female abdomen; they rotate dramatically during inflation of the basal hematodocha while the tips of the conductor and embolus are being inserted. These portions of the palp are obviously very *un*braced during copulation. The frequency of failed insertion attempts (flubs) was relatively high in *L. mariana* (just over 50% of all attempts). As mentioned above, in some cases the mispositioning of the palp of *L. mariana* was so substantial that the conductor and the embolus briefly engaged the wrong, ipsilateral side of the epigynum (the ancentral site of insertion - see below).

One puzzling pattern in the flubs of *L. mariana* was that they were more frequent later in copulation, while the very first insertion attempt of a copulation seldom failed. In contrast, "flubs" were most common at first in copulations of *Neriene litigiosa* (Keyserling 1886), and become rarer as the male gradually adjusted his position to that of the female (Watson 1991). It is possible that later insertions in *L. mariana* require more force or a more difficult orientation, or that females were more cooperative at first; but we were not able to discern that these were problems. Another possibility is that "flubs" is a misnomer, and that the behavior serves a stimulatory function as, for instance, may be the case for palpal "drumming" and "scrabbling" in *Nephila* (Robinson & Robinson 1973), and palpal scraping in *Schizocosa* Chamberlin 1904 species (Stratton et al. 1996). The association of

flubs with repositioning of the palp on the female's abdomen suggests, however, that in *L. mariana* flubs do indeed represent mistakes that the male attempts to rectify by repositioning his palp.

We can tentatively assign functional significance to several male genital structures and movements. The conductor hook (Fig. 11), represents the only external mechanical coupling structure of the palp. By lodging against the hood at the anterior edge of the atrium as the basal hematodocha is inflated, the hook serves to arrest the tips of the conductor and embolus at or near the entrance to the insemination duct, and may provide a brace allowing the embolus to be pushed into the female's insemination duct and/or the semen to be pushed into chamber I of the female's spermatheca. The movements of the tegulum against the paracymbium during insertion produce distal displacements of the base of the embolus that result in the insertion of the distal portion of the embolus into the female's insemination duct and spermatheca. The distance that the base of the embolus moved when it was displaced by the paracymbium was similar to the distance that the tip of the embolus projected beyond the tip of the conductor (Fig. 10). Judging by both their morphological relations and the synchronicity of their movements, the movement of the tegulum against the paracymbium was produced by inflation of the median hematodocha.

Transfer of encapsulated and thus immobile sperm to virgin females apparently occurs directly into the first chamber of the spermatheca during the long insertions at the beginning of copulation. Sperm were decapsulated, and thus became potentially mobile, in chamber I. There was additional material associated with sperm in various contexts, including the droplet before it was taken into the palps, in the sperm duct of the palp, in the white material that emerged from the palps during short insertions, in the chambers of the spermatheca, and in the epigynal plug. The origins and fates of these materials are not well understood. The uptake and ejection of the relatively viscous semen is probably produced by resorption and secretion of a material in the palp (Lamoral 1973; Suhm et al. 1995), and it seems likely that some of this material would be present in the lumen of the sperm duct along with the sperm, as has been seen in other spiders (Lamoral 1973; Suhm et al. 1995). Our observations are not sufficient to determine whether the plug material and the small spheres in the sperm fluid (Fig. 16) are the same material. Presumably the plugs serve to impede the access of other males to the female's internal genitalia.

Some of the material associated with sperm inside the female's spermathecae (Fig. 15) is probably derived from the female's spermathecal glands that empty via pores in the dorsal wall of the first spermathecal chamber, because this fluid was not present in the spermathecae of a female fixed soon after copulation, but was present in those of another fixed 21 min after copulation ended. Since only encapsulated sperm were found in the first female, while some sperm had become decapsulated in the other, this female-derived material may have a role in activating the sperm.

Our tentative suggestion that females may make critical contributions of material to the formation of epigynal plugs echoes similar female-active processes in other groups, such as the "insemination reaction" of *Drosophila* (Patterson 1947; Alonso-Pimentel et al. 1994). In apparent contrast with *Drosophila,* plug formation in *L. mariana* is highly variable. It may represent selective female cooperation with the reproductive interests of some males and not others.

**Taxonomic implications.**—The phylogenetic relations of *Leucauge* with metines, nephilines, and tetragnathines are still uncertain. *Leucauge* has often been included in the Metinae (Simon 1892; Kaston 1948), and linked to *Meta* (C.L. Koch 1836) and *Nephila* (Levi 1980). There are also two possible synapomorphies (dorsal femoral trichobothria, and posterior gut caeca) that link them with tetragnathines (Hormiga et al. 1995). Cheliceral clasping during copulation appears to provide another character supporting a close relationship with tetragnathines.

Cheliceral clasping occurs in *L. mariana* and *L. venusta* (Castro 1995; this study), *L. regnyi* (Alayon 1979 in Castro 1995) and three other unidentified *Leucauge* species (Eberhard 1994). It also occurs in *Plesiometa argyra* (Castro 1995; W. Eberhard unpubl.), and several species in the tetragnathine genera *Tetragnatha* and *Pachygnatha* (Gerhardt 1921, 1923, 1924a, 1928; Osterloh 1922;

Bristowe 1929; Levi 1981; Alvarez 1992; Preston-Mafham & Preston-Mafham 1993; W. Eberhard unpubl. on *T.* sp.). Cheliceral clasps do not occur in *Meta* or *Metellina* (Gerhardt 1921, 1927, 1928; Bristowe 1958) or *Nephila* (Gerhardt 1933; Robinson & Robinson 1973, 1980), nor do they occur in the outgroup Araneinae (e.g., Robinson & Robinson 1980). Thus cheliceral clasping may be a synapomorphy linking *Leucauge* and *Plesiometa* to *Tetragnatha* and *Pachygnatha.* The details of how clasping occurs vary in these groups. In contrast with *Leucauge* spp. and *P. argyra,* in which the female chelicerae open wide and seize those of the male, the males of *Tetragnatha pallescens, T. extensa,* and *T.* sp. wedge their chelicerae between those of the female, using a sexually dimorphic cheliceral tooth and a process on the antero-distal surface to (respectively) force the basal segments of the female's chelicerae apart, and to hold her fangs open (Bristowe 1929; Kaston 1948; Preston-Mafham & Preston-Mafham 1993). The clasp of *Pachygnatha clerki* is similar, but the male's kinked fangs also apparently press on and further restrict the movement of the female's fangs (Bristowe 1929). The male of *Pachygnatha degeeri,* in contrast, grasps the basal segments of the female's chelicerae with his, and holds them *closed.*

Two other derived characters supporting this same link between *Leucauge* and tetragnathines are the use of contralateral palps to inseminate the female (Huber & Senglet 1997), and the extraordinarily long pedipalpal trochanters (Fig. 10). Long pedipalpal trochanters are probably linked to cheliceral clasping, as they allow the basal portion of the male's palp to project ventrally, and thus avoid the possible obstacle posed by the female's chelicerae. Cheliceral clasping may have evolved before palpal trochanter elongation, with receptive females bending their abdomens ventrally to facilitate insertion. Or longer palps with long trochanters may have arisen first in groups without cheliceral clasps; long palps occur without cheliceral clasping in, for example, *Filistata* (Gerhardt 1923, 1933), theridiids in the genera *Theridium, Teutana, Steatoda* (Gerhardt 1924b, 1925, 1923, 1926), and the nesticid *Nesticus* (Huber 1993). In either case, the combination of cheliceral clasping and relatively long palps probably reduces the male's precision in positioning his palps just prior to insertion, because of the larger distance at which he must manipulate his palps (see below), and they may thus explain the origin of contralateral insertions. We observed high rates of flubs in *L. mariana* (above).

Our observations of how the palpal structures of *L. mariana* function provide additional links with tetragnathines. The homologous structures in *Pachygnatha clerki* (see Heimer 1982) work in the same way in several respects: 1) the tegulum moved against the paracymbium, 2) the hook of the paracymbium guides the rotation of the tegulum, and 3) this movement causes the embolus to be driven out of the conductor. In *Tetragnatha* sp. the paracymbium is also hooked into a groove on the tegulum (Huber & Senglet 1997; A. Senglet pers. comm.). In contrast, the paracymbium has no direct physical relation with the tegulum or the embolus in either the araneid *Araneus* (Grasshoff 1968), or in members of outgroups such as the linyphiids *Neriene* (van Helsdingen 1969), and *Mynoglenes* (Blest & Pomeroy 1978) and the nesticid *Nesticus* (Huber 1993).

Mating in *Leucauge* spp. occurs on the web rather than on a specially constructed mating thread that replaces web lines (Castro 1995; this study; W. Eberhard unpubl.), and the same is true in *Plesiometa* (Castro 1995; W. Eberhard unpubl.), *Nephila* (Robinson & Robinson 1973, 1980), and *Tetragnatha* (Preston-Mafham & Preston-Mafham 1993). In contrast, many araneines utilize a mating thread (Robinson & Robinson 1980; Robinson 1982). The lack of a mating thread is probably plesiomorphic, however (Robinson & Robinson 1978, 1980). The lack of tarsal rubbing by males during pre-copulatory courtship may also support a link with tetragnathines rather than araneines. This behavior is absent in *Nephila* and associated genera, and is widespread in araneines (Robinson & Robinson 1980) that are only distantly related (Scharff & Coddington 1997). It is not yet clear, however, whether tarsal rubbing constitutes a synapomorphy of this group.

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